Human Microbiomes: Challenging the Paradigm of a Sterile Uterus

Jeet Adhvaryu

Abstract
Microbiomes have been an integral part of life for millions of years. In humans, these bacterial colonies are found in the oral cavities, nasal cavities, the gastrointestinal tract, skin, and urogenital tract. These bacteria play an important role in digestion and protection. The placenta, however, is one location that has long been considered sterile. Bacterial presence was more often associated with inflammation, premature labour, preterm birth, and low birth weight. More recently, research has begun to suggest that a low abundance of bacteria in the placenta does not harm the fetus, and may be present more often than previously believed. These bacteria are very closely related to the maternal oral microbiome, and may arrive by hematogenous transmission through chorionic villi. Through a discussion and review of the literature up until now, it is clear that there is much uncertainty in this new field. No doubt, future research may focus on elucidating the method of hematogenous bacterial transmission to the placenta and furthering our knowledge on the characteristics and relationship of these bacteria and the maternal environment.

Keywords
Microbiota — Placenta — Preterm Birth — Microbiome — Sterile Uterus — Chorioamnionitis — Human Microbiome Project

1. Introduction
Microbiomes, bacterial colonies in specific body site niches, have been a part of life for millions of years (Aagaard et al., 2014). These microbiomes exist in places such as the oral and nasal cavities, the gastrointestinal tract, skin, and urogenital tract (Huttenhower et al., 2012; Moloney et al., 2014). They are characterized of commensal or mutualistic bacteria that serve to protect or benefit the body—some by preventing colonization of more harmful species, and some by aiding in processes such as digestion and growth (Aagaard et al., 2012; Moloney et al., 2014). Dysbiosis—a microbial imbalance—may result in a multitude of disorders, such as inflammatory bowel disease, allergic diseases, obesity, metabolic syndrome, and heart disease (Barrett et al., 2013; Boyle et al., 2011; Lahtinen et al., 2009; Moloney et al., 2014).

The Human Microbiome Project (HMP) Consortium has recently been the first to study and estimate the structure, function, and diversity of healthy human microbiomes throughout the body (Huttenhower et al., 2012). Although bacteria have been found to be beneficial, it has long been believed that one region, the placenta, was a sterile environment in which the fetus must grow without bacterial contamination (Aagaard et al., 2014; Jiménez et al., 2008; Wassenaar & Panigrahi, 2014). Bacterial presence was more often associated with inflammation from immune response, premature labour, preterm birth, and low birth weight (Groer, Gregory, Louis-Jacques, Thibeau, & Walker, 2015; Jiménez et al., 2008; Wassenaar & Panigrahi, 2014).

Recent research suggests, however, that a low abundance of bacteria are present in the placenta that do not harm the fetus (Aagaard et al., 2014; Ardissone et al., 2014). The pathogenic nature of bacteria may not be a trait to any individual bacteria, but rather an immune reaction to a high abundance of any bacteria (Aagaard et al., 2014). This presence of bacteria that is not harmful has drawn a lot of attention, and raises new questions on the time of development of the human microbiome. Here we discuss human microbiomes and preliminary evidence that the presence of a fetal microbiome is not inherently harmful; though results are currently inconclusive, assisting in the development of a healthy gut microbiome earlier than birth remains plausible. We will form a strong fundamental understanding of the nature of bacteria and microbiomes, the placenta, and intrauterine infection. Thereafter, current frontiers of research on fetal microbiomes and possible antenatal treatments will be investigated.

2. An Overview of Bacterial Microbiomes
Microbial communities can be complex, and are often strongly reliant on their environments, which include other species of bacteria. An example of this is in the human oral cavity, where Streptococcus spp. and Actinomyces spp. attach to the salivary pellicle coatings of teeth. These initial founding bacteria form a substrate that allows for other bacteria, such as Fusobacterium nucleatum, to colonize thereafter (Mark-
Welch, Rossetti, Rieken, Dewhirst, & Borisy, 2016). Similarly, facultative anaerobes such as enterobacteria and staphylococci consume oxygen in the gastrointestinal tract of newborns, and allow for more strict anaerobes, like *Bifidobacterium*, to proliferate. Each new colonizer has an effect on future colonization and on already established bacteria.

The human microbiome is known to begin forming at birth, at the latest (Barrett et al., 2013; Moloney et al., 2014). Neonates delivered vaginally show initial colonization by *Lactobacillus, Prevotella*, or *Sneathia* spp., bacteria commonly associated with the vaginal microbiome, and neonates delivered through Caesarean-section (C-section) show a dominance of *Staphylococcus, Corynebacterium*, and *Propionibacterium* spp., which resemble the microbiome of the mother’s skin (Barrett et al., 2013; Moloney et al., 2014; Weyrich, Dixit, Farrer, Cooper, & Cooper, 2015). It would be inaccurate to state that these initial founders are the same bacteria that will form the remainder of the infant’s microbiome, because several factors, such as diet, stress, infection, and environment will keep the microbiome at a dynamic equilibrium throughout life (Barrett et al., 2013; Huttenhower et al., 2012; Moloney et al., 2014).

The HMP Consortium were some of the first to conduct a thorough study of the bacterial species and diversity at different microhabitats on the human body—up to 18 body sites were investigated in varying locations of the gut, oral cavity, skin, and vagina (Huttenhower et al., 2012). The diversity of microbes at body sites has been linked to many human diseases, such as inflammatory bowel disease (low diversity in the gut), or bacterial vaginosis (high diversity in the vagina). Body site diversity can be defined as alpha and beta diversity (Barrett et al., 2013; Huttenhower et al., 2012). Alpha diversity is within subjects, and beta diversity is in the same habitat among different subjects. Saliva, for example, has some of the highest alpha diversities, but one of the lowest beta diversities (Huttenhower et al., 2012). Beta diversity of bacteria to the fetus (Aagaard et al., 2014). Although bacterial cultures and various other methods are still used, 16S sequencing has become the most common due to the gene’s presence in virtually all bacteria, the relative novelty of the plausibility, for this paper the gastrointestinal microbiome will not be a focus.

There are three particularly important maternal microbiomes to consider when discussing bacterial transfer to the fetus and neonate: vaginal, epidermal (skin), and oral (Aagaard et al., 2012, 2014; Ardissone et al., 2014; Boyle et al., 2011; Huttenhower et al., 2012; Jiménez et al., 2008; Linhares et al., 2013). These are particularly important because of the likelihood of bacterial transference to the fetus, and their impact on the overall development to the establishment of microbiomes in the fetus. The vaginal and epidermal bacteria will transfer depending on whether the delivery is vaginal or through C-section (Barrett et al., 2013; Moloney et al., 2014). The oral microbiome has recently been implicated in transfer of bacteria to the fetus (Aagaard et al., 2014).

The most common way that bacteria in the discussed regions are identified, is through 16S rRNA gene sequencing. Although bacterial cultures and various other methods are still used, 16S sequencing has become the most common due to the gene’s presence in virtually all bacteria, the stability of the gene’s function over time, and the size of the gene (1,500 base pairs) (Janda & Abbott, 2007). The majority of the results of the bacterial characterizations that follow are a product of the
3. Vaginal Microbiome

The human vagina is host to many different species of bacteria, most of which are lactic acid-producing bacteria, generally of the Lactobacillus genus (Linhares et al., 2013). The most common Lactobacillus species include L. crispatus, L. iners, L. gasseri, and L. jensenii (Linhares et al., 2013). These are not always the dominant species in healthy women, since it has been reported that Black and Hispanic women often have other bacteria present that result in a lower overall pH of the vagina, though no detriment to health (Linhares et al., 2013).

Aagaard et al. (2012) found, however, that there are significant variations in the pregnant and non-pregnant vaginal microbiome. The hierarchy of most dominant to least dominant order remained constant: Lactobacillales was the most dominant, followed by Clostridiales, Bacteroidales, and Actinomycetales. However, species richness was significantly reduced, indicating that a factor not investigated in their study had a strong effect during pregnancy.

4. Oral Microbiome

The oral microbiome has the most diverse array of bacteria, with 707 species present in the Human Oral Microbiome Database (HOMD) (Mark-Welch et al., 2016). Dewhirst et al. (2010) contributed greatly to the provisional naming of species and phylotypes in the HOMD; approximately 400 novel taxa were added, and the HOMD stands to be the first curated characterization of the human oral microbiome. A total of 13 phyla have thus far been observed in the oral microbiome: Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Euryarchaeota, Firmicutes, Fusobacteria, Proteobacteria, Spirochaetes, SR1 (Sulfur River 1), Synergistetes, Tenericutes, and TM7 (Candidatus Saccharibacteria) (Dewhirst et al., 2010). The full intricacies of the phylogenetic trees lie beyond the scope of this review, but the value of such work is worth note.

In one study, Mark-Welch et al. (2016) found that most species in the database were habitat specialists; if only one particular oral habitat was considered, such as plaque, the number of species is greatly reduced. In supragingival plaque (SUPP), 57 genera were detected. Of those 57 genera, only 13 had a 3% prevalence or more—a level of prevalence consistent among samples. Furthermore, those 13 genera accounted for 85% of the sequenced data in SUPP.

On identification of the bacteria in SUPP by Mark-Welch et al. (2016), an organization labelled “hedgehog” was observed. Hedgehog consisted of a mass of Corynebacterium filaments at the base, and Streptococcus on the outer layers. These structures also had a few taxa that were consistently present, mainly Porphyromonas, Haemophilus, Aggregatibacter, Neisseriacaeae, Fusobacterium, Leptotrichia, Capnocytophaga, and Actinomyces—along with the aforementioned Corynebacterium and Streptococcus.

While SUPP is only one example, it serves as a model for other oral regions. There are abundant, foundational taxa, such as Corynebacterium, and others colonize through the subsequent facilitation.

5. Skin Microbiome

There are up to one billion bacteria in a given square centimeter of human skin, giving some insight on their abundance (Weyrich et al., 2015). These bacteria play a vital role in processes such as disease prevention. Staphylococcus aureus infection, for example, is prevented by some species that hydrolyse sebum lipids into toxic fatty acids (Weyrich et al., 2015). Dysbiosis, as with other microbiomes, does lead to diseases such as dermatitis, psoriasis, and acne (Weyrich et al., 2015). Of all the human body sites investigated by the HMP (oral, skin, gut, and vaginal regions), the skin had the highest beta diversity (Huttenhower et al., 2012; Weyrich et al., 2015).

A thorough review of the skin microbiome was conducted by Grice & Segre (2011) and discussed the several factors that contribute to the diversity of the microbiome. These factors include topography (whether the skin is moist, dry, or sebaceous) the environment, age, geographic location, and sex (Grice et al., 2009; Grice & Segre, 2011). For example, the face and chest contain a high density of sebaceous glands, and thus lipophilic bacteria, such as Propionibacterium sp. and Malassezia spp., are more likely to be found there (Grice & Segre, 2011). Moist sites such as the gluteal crease or the sole of the foot have been shown to have a greater abundance of Staphylococcus and Corynebacterium spp. (Grice & Segre, 2011). Dry skin has been found to be the most diverse, with no one phyla dominating (Grice & Segre, 2011). In general, four phyla have been found dominant—Actinobacteria (52%), Firmicutes (24%), Proteobacteria (17%), and Bacteroidetes (7%) (Grice et al., 2009; Weyrich et al., 2015).

The skin microbiome is significantly less diverse is neonates, acquiring only the bacteria from contact during birth as mentioned before. Capone et al. (2011) found that unlike the adult microbiome, the infant microbiome is predominated, in decreasing abundance, by Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes. Furthermore, the relative abundance of bacterial communities (or species evenness) increased significantly from 1-3 months of age to 7-12 months (Capone et al., 2011). These variations in the infant microbiome may also be attributed to differences in topography; the infant skin is more retentive of water, and thus can be compared to the moist sites in an adult (Capone et al., 2011). Indeed, the Staphylococcus genus found in adult moist skin sites is in high abundance throughout the infant skin (Capone et al., 2011).

The bacteria thereafter are strongly affected by the environment the neonate comes in contact with (Hendricks-Muñoz et al., 2015). Large shifts in bacteria can occur early in life, and changes continue to occur as surfaces develop distinct moisture and temperature characteristics (such as underarms), and as individuals reach puberty (Hendricks-Muñoz et al.,...
Diseases such as atopic dermatitis and acne have identified the microbial components that are likely involved, but the causes of these shifts to detrimental bacteria remain elusive (Weyrich et al., 2015). The prevalence of acne, for example, is as high as 80% in the majority of Western countries but it is rare in hunter-gatherer societies (Weyrich et al., 2015). These findings indicate that there is much research still to be done on the skin microbiome.

6. The Placenta

How the aforementioned bacteria from their respective microbiomes could reach a growing fetus can be understood after considering the anatomy and physiology of placentation. The placenta is unique, in that allogeneic cells are tolerated by the mother (Reece et al., 2011; Saladin, 2015). The degree of uterine invasion by the trophoblast cells of the blastula (one of the early stages of fetal development, before uterine implantation) varies in animals from epitheliochorial placentation, a very superficial form of invasion, to haemochorial placentation, where the trophoblast cells come into direct contact with the mother’s blood in her vessels (Moffett & Loke, 2006). Humans undergo haemochorial placentation.

The placenta is comprised of two sections, the Chorion frondosum (chorion) and the Decidua basalis (decidua); both are separated by the basal plate (Wassenaar & Panigrahi, 2014). The chorion genetically belongs to the fetus and is formed from the trophoblast cells that thicken and extend chorionic villi into the surrounding maternal tissue (Reece et al., 2011; Wassenaar & Panigrahi, 2014). The decidua genetically derives from the mother. Following endometrial attachment and penetration by trophoblast cells, the stroma differentiates due to the effect of hormones and cell signalling, forming the decidua (Aplin, 1991; Bowen et al. 2002; Moffett & Loke, 2006; Reece et al., 2011). Spiral arteries form the blood supply of the full structure, and are dilated by the action of the invading trophoblasts (Pijnenborg, Vercruysse, & Hanssens, 2006). The maternal portion has a higher blood pressure than the fetal portion, which allows for the exchange of gasses, nutrients, and waste by bathing the chorionic villi in blood from the spiral arteries (Geusens et al., 2008; Pijnenborg et al., 2006; Wassenaar & Panigrahi, 2014). Fluid itself, however, is not exchanged (Wassenaar & Panigrahi, 2014).

7. Complications and Chorioamnionitis

Bacterial presence in the uterus is not regarded detrimental without merit. For example, preterm births have been a problem for obstetricians, and over the last several years rates have been increasing significantly (Bersani, Thomas, & Speer, 2012). Inflammation of the chorioamniotic membranes, chorionic plate, umbilical cord, and amniotic fluid caused by bacterial infection (chorioamnionitis), is a major risk factor for preterm birth and complications, with approximately 25% of preterm births related to intrauterine bacterial infection (Bersani et al., 2012; Wassenaar & Panigrahi, 2014).

Labour in humans always involves an inflammatory response, regardless of gestational age. This is usually a result of prostaglandins and cytokines, which have been initiated by physiological factors such as corticotrophin-releasing hormone and pathological processes (Steel et al., 2005; Steinborn, Gunes, Roddiger, & Halberstadt, 1996). The mechanisms for infection induced labor and normally occurring labor are very similar, despite separate root causes as shown in figure 3 (Bovew et al., 2002; Steinborn et al., 1996). Preterm labour at 23-32 weeks of gestation is most often associated with bacterial infection in the uterus (Steel et al., 2005). The association between chorioamnionitis and preterm birth is strong enough that intrauterine infection is often being diagnosed without the determination of a bacterial presence (Steel et al., 2005; Steinborn et al., 1996).

It is generally accepted that intrauterine infections originate from the maternal urogenital tract (Wassenaar & Panigrahi, 2014). The bacteria infiltrate the chorion and decidua and incite the inflammatory response—this can further lead to a crossing of the amniotic barrier and an infection of the amniotic fluid (Bowen et al., 2002). A less common haematogenous method of infection has also been suggested, in which a cascade of inflammatory cytokines from the maternal portion of the placenta invade the amniotic fluid through the chorion (Bowen et al., 2002; Wassenaar & Panigrahi, 2014).

Associations have long been drawn between periodontal diseases and birthing complications. Preliminary evidence began to support the association with the increased prevalence of genitourinary tract infections with complications such as preterm birth and low birth weight (Offenbacher et al., 1996). Offenbacher et al. (1996) initially studied the association between periodontal disease and preterm low birth weight (PLBW). Using a fairly coarse measurement of periodontal disease (mean clinical attachment levels), significance was still found between women with no sign of periodontal dis-
Figure 3. A flowchart constructed by Bowen et al. (2002) depicting the mechanisms of birth. Cytokines play a vital role in the onset of birth, and they are initiated by both labour and ascending uterine infection.

Figure 4. Results of the Bray-Curtis (B-C) dissimilarity assessment acquired from Aagaard et al. (2014). Data for the gut, vagina, posterior auricular skin, and nasal airways was obtained from the HMP, while the remainder was through the authors’ own research. Thicker connecting lines indicate greater similarity, while no connections indicate a lack of significant similarity.

8. Fetal Microbiome and Origins

It is clear by now that bacterial invasion on the uterus has been considered detrimental for several well supported reasons. Two years ago, however, the fetal microbiome was intricately interrogated by Aagaard et al. (2014), and the species were identified in a study. Associations were also drawn between the fetal microbiome and places of origin. The majority of taxa found in the placenta using DNA based techniques represented non-pathogenic commensal bacteria from the Firmicutes, Tenericutes, Proteobacteria, Bacteroides, and Fusobacteria phyla. Oral commensal species, such as *E. coli*, held the highest abundance in most individuals. *Prevotella tannerae* and non-pathogenic *Neisseria* spp., both from the oral microbiome, were also found. Performing a Bray-Curtis dissimilarity test using known skin, vaginal, gut, stool, oral, and the newly identified placental microbiomes, it was found that the placental bacteria were significantly related to oral bacteria. Bacteria found in the tongue, tonsils, saliva, and throat were all strongly related to placental bacteria; figure 4 graphically demonstrates these findings. Aagaard et al. (2014) further found that the abundance of this bacteria was correlated with gestational age at delivery. This supports the previous paradigm of a high abundance of bacteria viewed as detrimental due to the inflammatory response, but also proposes the possibility of a low abundance, harmless bacterial presence.

Although a prominent study, Aagaard et al. (2014) was not the first to suggest a non-pathogenic bacterial presence in the uterus. Jiménez et al. (2008) conducted a study on meconium of healthy neonates. Meconium is a good indicator of bacteria present in the placenta, due to the constant swallow-
ing of amniotic fluid during gestation (Ardissone et al., 2014; Jiménez et al., 2008). If the paradigm of a sterile placenta was true, then the meconium should also be sterile. Meconium was collected with the criteria that no probiotics were given during gestation, the neonate had not yet breast fed, and the meconium was evacuated within the first 2 hours of birth. Enterococci was present in 80% of the sample, and Staphylococci were present in 52%; E. fecalis and S. epidermidis were the predominant species respectively. Several other species were identified in lower abundance, and each meconium had anywhere between 1 and 5 bacterial species.

To further investigate the mechanisms by which these bacteria entered the placenta, (Jiménez et al. (2008) orally inoculated pregnant mice with labelled E. f. cie. Mice were dosed daily from the first day of mating till conception. The labelled E. f. cie. was subsequently observed in the meconium of newly born mice.

Many studies suggest that these bacteria likely arrived from the maternal oral cavities, and cite hematogenous transmission (Aagaard et al., 2014; Jiménez et al., 2008; Prince et al. 2014). The exact method by which this hematogenous transmission is conducted, is not entirely known. The immunological environment in the placenta is unique in order to prevent rejection of the fetus. Increased levels of the immunosuppressive cytokine, IL-10, as well as the infection fighting IL-6, are found in the amniotic fluid (Prince et al., 2014). It is suggested that this combination facilitates energy harvest of the fetus from the mother by altering the mother’s microbiome; this alteration may also increase the placenta’s susceptibility to hematogenous colonization (Prince et al., 2014).

One suggested mechanism of hematogenous transfer is that due to the chorionic villi being in direct contact with maternal blood, bacteria ingested orally and reaching the digestive tract survive to reach the villi—along with the cytokines that they have activated (Bowen et al., 2002; Dasanayake, Li, Wiener, Ruby, & Lee, 2005). This cascade of cytokines cause inflammation of the chorion and facilitate the entrance of bacteria into the placenta. However, this is a more accurate model of chorioamnionitis, and does not fully explain the presence of non-pathogenic bacteria.

9. Health Implications
With this fairly recent discovery on the presence of bacteria in the placenta, one can predict that this opens up many possibilities in terms of neonate health. It is possible that antenatal interventions could have more profound long term effects. One study by Lahtinen et al. (2009) suggests that administration of a probiotic, Lactobacillus rhamnosus GG (LGG), can influence intestinal colonization of specific Bifidobacterium species. Infants colonized by B. adolescens and species typical of adult intestinal microbiota are reported to have allergic diseases, while those colonized by the B. longum group and B. breve were not (Lahtinen et al., 2009). Results showed that ingestion of probiotic pills starting at 36 weeks of gestation up till birth, significantly altered Bifidobacterium species in infants 90 days after delivery. Infants were more likely to be colonized by the B. longum group if probiotics were administered (Lahtinen et al., 2009).

Soon after, a similar experiment was conducted by Boyle et al. (2011), in which probiotic supplementation was administered in the same way to pregnant women. Oddly, despite both studies having a good sample size (122 women in 2009, and 250 in 2011), they obtained contradictory results. Boyle et al. (2011) found that there was no significant association of probiotic treatment with allergic disease prevention or bacterial species alteration.

Neither study understood the mechanism behind the predicted effect of LGG, but made their predictions based on somewhat positive results in prior studies (Boyle et al., 2011; Lahtinen et al., 2009; Matsu, Watanabe, Tanaka, Fukuda, & Oyaizu, 1999). The heterogeneity of these results attest to the limitations of our current knowledge in the field of fetal microbiomes.

10. Conclusions
The presence of a low-abundance, non-pathogenic microbiome in the placenta has been an exciting discovery, and the current knowledge base has even been able to narrow down the places of origin for the bacterial species. However, it is important to note that this does not imply that bacteria are not harmful to the fetus. Aagaard et al. (2014) noted that despite the presence of bacteria in healthy newborns, there were still significant associations with altered microbiomes and high abundances of bacteria with preterm birth. There is no doubt that future research should focus on the implications of this newly identified microbiome, but research conducted by Boyle et al. (2011) and Lahtinen et al. (2009) are a testament to the uncertainty that currently lies in this new field. Furthermore, much research points to the transfer of oral bacteria hematogenously to the fetus, but there is not enough research on the mechanism behind this transfer aside from the assumption that it is due to transfer to the chorionic villi and some form of cascade of cytokines.

No doubt, future research will focus on these short comings and perhaps assisting in the development of an ideal set of microbiomes in a newborn will become possible. This line of research may also assist in the reduction of preterm births due to chorioamnionitis from prevention or direct alteration.

11. References


